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Reduction of elevated plasma globotriaosylsphingosine in patients with classic Fabry disease following enzyme replacement therapy

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ABSTRACT

Fabry disease is treated by two-weekly infusions with α-galactosidase A, which is deficient in this X-linked globotriaosylceramide (Gb3) storage disorder. Elevated plasma globotriaosylsphingosine (lysoGb3) is a hallmark of classical Fabry disease. We investigated effects of enzyme replacement therapy (ERT) on plasma levels of lysoGb3 and Gb3 in patients with classical Fabry disease treated with agalsidase alfa at 0.2 mg/kg, agalsidase beta at 0.2 mg/kg or at 1.0 mg/kg bodyweight. Each treatment regimen led to prominent reductions of plasma lysoGb3 in Fabry males within 3 months ($P=0.0313$), followed by relative stability later on. Many males developed antibodies against α-galactosidase A, particularly those treated with agalsidase beta. Patients with antibodies tended towards smaller correction in plasma lysoGb3 concentration, whereas treatment with high dose agalsidase beta allowed a reduction comparable to patients without antibodies. Pre-treatment plasma lysoGb3 concentrations of Fabry females were relatively low. In all females and with each treatment regimen, ERT gave reduction or stabilisation of plasma lysoGb3. Our investigation revealed that ERT of Fabry patients reduces plasma lysoGb3, regardless of the recombinant enzyme used. This finding shows that ERT can correct a characteristic biochemical abnormality in Fabry patients.

1. Introduction

The lysosomal storage disorder Fabry disease (OMIM 301500) results from deficient activity of the lysosomal hydrolase α-galactosidase A (EC 3.2.1.22) [1,2]. Since the α-galactosidase A (*GLA*) gene is located on Xq22.1, hemizygous males suffer from Fabry disease. However, many female heterozygotes also display symptoms despite considerable amounts of circulating residual enzyme that varies due to random X-inactivation [3,4]. In both males and females different phenotypic manifestations of Fabry disease occur [2]. The classical manifestations in males involve angiokeratoma, anhidrosis and acroparesthesias at puberty, followed by renal insufficiency, left ventricular hypertrophy (LVH) and cerebrovascular disease in the fourth or fifth decade of life. In females the disease manifestations are usually, but not always, attenuated. A complete lack or severely reduced α-Gal A activity results in classical manifestations of Fabry disease. During the past decades α-Gal A deficient patients with relatively few and isolated symptoms, such as LVH or renal failure at later stages in life, have been described [2,5,6]. These patients are generally referred to as atypical Fabry patients, to distinguish them from those with multi-organ symptoms at a young age. Relatively high residual α-Gal A activity is often noted in these atypical cases [2]. The precise causes for the remarkable heterogeneous manifestations of Fabry disease are still poorly understood [5,7–9]. The nature of mutations in the *GLA* gene and the corresponding degree of residual α-Gal A activity, undoubtedly influence disease manifestations [10–12]. There is growing evidence for other modifiers beyond the *GLA* gene. For example, a modulating role may be envisioned for α-galactosidase B, another α-galactosidase arisen by gene duplication with low activity towards the lipid substrate of α-Gal A [13,14]. In addition, polymorphisms c.174G>C of interleukin-6, c.894G>T of endothelial nitric oxide synthase (eNOS), factor V c.1691G>A mutation (factor V Leiden), and the c.13A>G and c.79G<A of protein Z are all significantly associated with the presence of cerebral white matter lesions on brain MRI [15]. Two polymorphisms in the

Abbreviations: eNOS, endothelial nitric oxide synthase; ERT, enzyme replacement therapy; Gb2, galabiosylceramide; Gb3, globotriaosylceramide; *GLA* gene, α-galactosidase A gene; LVH, left ventricular hypertrophy; lysoGb3, globotriaosylsphingosine; OPA, o-phtaldialdehyde

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NOS3 gene, encoding eNOS, have also been shown to influence hypertrophic cardiomyopathy as measured by left posterior wall thickness of the heart [16].

Deficiency of α-Gal A results in accumulation of its glycosphingolipid substrates in lysosomes of endothelial, perithelial, and smooth muscle cells of the vascular system, as well as renal epithelial cells, myocardial cells, and cells of the autonomic nervous system [17]. The accumulating glycosphingolipids contain terminal α-galactosyl moieties, such as globotriaosylceramide (Gb3; also named ceramidetrihexoside); galabiosylceramide (Gb2); and, to a lesser extent, blood group B, B1, and P_1 antigens [2,18]. The prominent lysosomal storage of Gb3 in cells of the arterial wall is generally thought to cause a systemic vasculopathy with progressive renal insufficiency, cardiac involvement and CNS pathology in Fabry patients [2,9,19]. Symptomatic Fabry hemizygotes, but not most heterozygotes, show increased plasma Gb3 levels [2,4]. Mass spectrometry-based and HPLC-based procedures have become available allowing accurate quantification of the globoside [20–24]. Plasma Gb3 has been found to poorly reflect Fabry disease manifestations and therapeutic outcome [4,25–27]. Recently, another striking lipid abnormality has been noted in patients with classical Fabry disease manifestations [28]. Globotriaosylsphingosine is deacylated globotriaosylceramide (designated as lysoGb3). The relative increase in the plasma lysoGb3 exceeds that of Gb3 by more than an order of magnitude. High nanomolar lysoGb3 concentrations occur in plasma samples from symptomatic Fabry males with classical disease manifestations. Also in symptomatic Fabry females, clearly increased levels of lysoGb3 occur whilst concomitantly Gb3 concentrations are in the normal range [28].

Two different recombinant α-Gal A preparations are in use for the treatment of Fabry disease [29,30]. One enzyme is produced using Chinese hamster ovary cells with classic recombinant technology (agalsidase beta, Fabrazyme) and the other enzyme is produced using cultured human skin fibroblasts with an activated promoter of the α-gal A gene (agalsidase alfa, Replagal). Both recombinant enzymes are comparable in properties and differ only slightly in glycan composition [31]. The two enzyme preparations have independently been examined in clinical investigations and are both registered in Europe for the treatment of Fabry patients. Although both enzyme replacement therapies (ERTs) were found to result in the desired clearance of Gb3 from the endothelium, the clinical effects are not as robust as anticipated based on the impact of ERT in type 1 Gaucher disease [32]. In some patients stabilization of renal function and improvement in cardiac hypertrophy occurs upon therapy, but a considerable number experience progressive complications [33]. From a recent analysis of the Fabry Outcome Survey observational database, it was concluded by comparison with historical natural history data for patients who were not treated with ERT, that long-term treatment with agalsidase alfa leads to substantial and sustained clinical benefits [34]. However, discerning the specific effect of ERT from that of other interventions, for example the use of renin–angiotensin inhibitors, remains a challenge [35]. It has been documented that a large portion of Fabry hemizygotes develops antibodies against the recombinant enzymes during ERT [36–38]. In addition, it has been hypothesized that the formation of antibodies possibly affects outcome [39,40].

Since monitoring of efficacy of ERT is hampered by the heterogeneous manifestations of Fabry disease as well as the irreversible nature of many symptoms, we investigated the impact of therapy on plasma lysoGb3 levels in patients with classical Fabry disease manifestations. Plasma lysoGb3 can be accurately quantified and offers an objective parameter to assess whether the underlying biochemical deficiency in Fabry patients is corrected by therapy. In a previous study, we investigated outcomes of Gb3 levels in relation to different ERTs [38]. We here report the outcome of the investigation in which plasma Gb3 as well as lysoGb3 levels were monitored in patients receiving either agalsidase alfa (0.2 mg) or agalsidase beta (0.2 or 1.0 mg) per kg bodyweight every 2 weeks. The impact of formation of neutralizing anti-(α-Gal A) antibodies on plasma lipid corrections is also described.

2. Materials and Methods

2.1. Classical Fabry disease patients and controls

Control subjects for Gb3 analysis consisted of 10 males and 15 females. Plasma lysoGb3 levels were measured in 9 male and 9 female healthy volunteers (age range 5–77 years). Forty-three Fabry patients (22 males and 21 females; age range 18–71 years) from the Academic Medical Center (AMC) and from the Universitätsklinikum Würzburg (UKW) who presented with classical Fabry disease and received a minimum of 12 months of ERT were eligible for this study. This is a subset of patients described in an earlier study [38] excluding patients with an atypical variant of disease and with incomplete sample collection. All patients were treatment naïve prior to enrolment. Classical Fabry disease is defined by the presence of characteristic symptoms and elevated plasma lysoGb3 and/or Gb3 in hemizygotes, and in case of heterozygotes, having a mutation known to be associated with a classical phenotype (through literature or a male family member with classical symptoms). Patients received three different ERT treatments: infusions every two weeks with agalsidase alfa at 0.2 mg/kg ($n=14$; 7 males and 7 females), agalsidase beta at 0.2 mg/kg ($n=11$; 6 males and 5 females), or agalsidase beta at 1.0 mg/kg ($n=18$; 9 males and 9 females). All patients had been diagnosed by means of a reduced α-Gal A activity in leukocytes (males) and/or a mutation in the α-Gal A gene (females) [33]. None of the investigated male Fabry patients showed a residual enzyme activity exceeding 5% of the normal mean. Stored blood samples from Fabry disease patients were analyzed. Informed consent and approval of the institutional review board had been obtained as part of a previous study [38].

2.2. Antibodies

Serum samples were evaluated for the presence of anti-(α-Gal A) antibodies as previously described [36]. To assess neutralizing activity, different volumes of serum (0.1–10 μl of serum, corresponding to a dilution of 1/150–1/1.5) were incubated with a standard amount of recombinant α-galactosidase A (agalsidase beta, 2.1 ng). Enzyme activity was determined after 10 minutes of incubation at room temperature. The serum dilution that resulted in 50% reduction of the enzyme activity was recorded (IC50). Assuming a linear relationship, the amount of enzyme that would be inhibited by 1 μl of serum was calculated.

2.3. Plasma Gb3 and lysoGb3

Plasma Gb3 and lysoGb3 were determined at $t=0$, $t=3$ and $t=12$ months of ERT. If available, lipid concentrations were also measured in plasma specimens obtained after 6 and 9 months of treatment. Quantitation of Gb3 in plasma samples was performed as described previously [24]. Quantitative measurements of plasma lysoGb3 were performed as described with minor modifications [28,41]. Briefly, 100 μl of plasma was extracted with 600 μl of chloroform/methanol 1/2 (vol/vol). The extract was centrifuged for 10 min at 14,000*g* and the pellet was discarded. To the supernatant, 500 μl chloroform/MQ-H_2O 1/1.5 (vol/vol) was added, mixed, and centrifuged for 2 min at 14,000*g* to separate the phases. The upper phase was collected, and the lower chloroform phase was re-extracted with 500 μl of methanol/ MQ-H_2O 1/1 (vol/vol) to quantitatively extract all lysoGb3. The combined upper phases were dried under nitrogen flow, taken up in 500 μl MQ-H_2O, and extracted twice with 500 μl of water-saturated 1-butanol. LysoGb3 was recovered from the butanol phase with an overall recovery of >90%. The butanol phase was dried, dissolved in 120 μl (hemizygotes) or 90 μl (heterozygotes) of freshly prepared 0.1 M NaOH in methanol and incubated at 37 °C for 1 h. Of this solution, 50 μl was derivatized with 25 μl of o-phtaldialdehyde (OPA) reagent (5 mg of OPA, 100 μl of ethanol, 5 μl of 2-mercaptoethanol, and 10 ml of 3% boric acid, pH 9.0). The OPA-derivatized lysoGb3 was separated by HPLC and quantified by

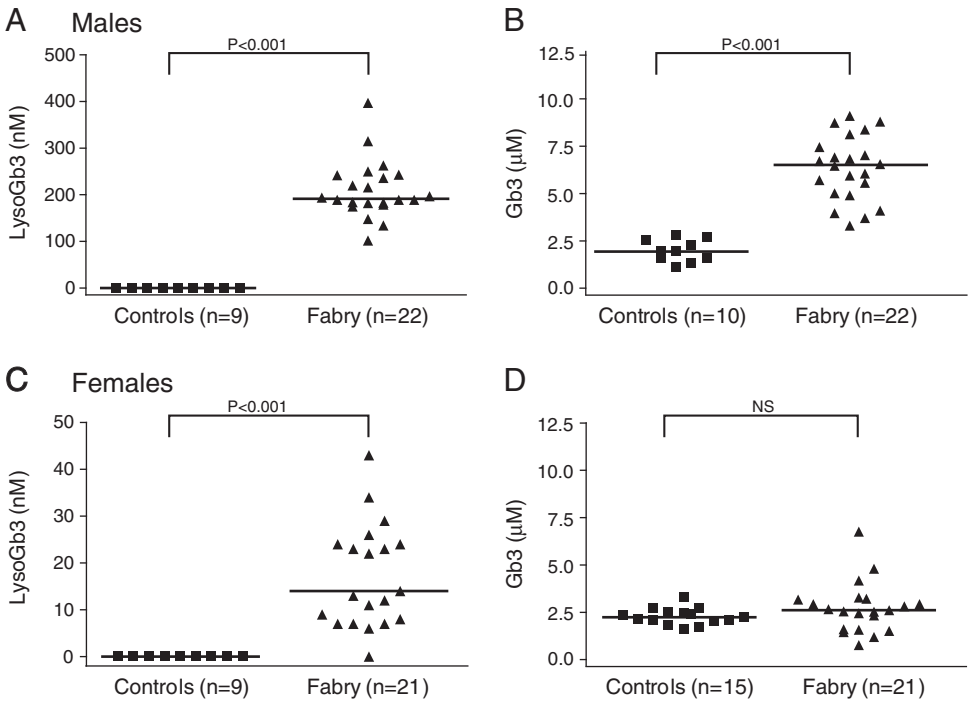

Fig. 1. Plasma levels of lysoGb3 and Gb3 in controls and Fabry patients prior to therapy. (A) Plasma lysoGb3 levels in male control subjects ($n=9$) and male Fabry patients ($n=22$). (B) Plasma Gb3 levels in male control subjects ($n=10$) and male Fabry patients ($n=22$). (C) Plasma lysoGb3 levels in female control subjects ($n=9$) and female Fabry patients ($n=21$). (D) Plasma Gb3 levels in female control subjects ($n=15$) and female Fabry patients ($n=21$). Lipid levels were determined as described in Materials and methods. The horizontal line represents the median value in each group. *P*-values (two-tailed Mann–Whitney rank sum test) are indicated when subgroups were statistically different. NS, not significant.

fluorescence detection as described previously [24]. All plasma samples were extracted in duplicate. Quantification was performed by addition of lysoGb3 (Sigma-Aldrich) to normal plasma at concentrations ranging from 0 to 1 mM. The limit of detection of plasma lysoGb3 is 3 nM.

2.4. Statistical methods

Results are given as median and range. Differences in lipid levels between groups were assessed by the Mann–Whitney *U*-test (2 groups) and the Kruskal–Wallis test (>2 groups). To compare lipid levels between patients before and after therapy, data were analyzed using the Wilcoxon matched pairs test (2 time points). To compare lipid levels between patients before and after one year of therapy, data were analyzed using repeated measures analysis of variance (>2 time points). For this analysis lipid data of individuals were expressed as percentage of $t=0$ (pre-treatment). Correlations were tested by the rank correlation test (Spearman coefficient, ρ). Results were considered to be statistically significant when two-tailed *P*-values were <0.05.

3. Results

3.1. Pre-treatment plasma concentrations of lysoGb3 and Gb3

Patients with classical manifestations of Fabry disease receiving ERT for a minimum period of one year were analyzed on plasma concentrations of lysoGb3 and Gb3. All patients (22 males and 21 females) showed increased plasma lysoGb3 levels prior to ERT (Fig. 1). One female patient formed an exception, having a very low lysoGb3 level. All males showed elevated plasma Gb3, but only in 3 females the concentration of Gb3 was above the normal range (Fig. 1).

3.2. Effect of different ERT regimens on plasma lipids in hemizygotes

Fig. 2 shows the effect of ERT on lysoGb3 and Gb3 levels for hemizygotes. Plasma lysoGb3 and Gb3 concentrations of male patients were not significantly different at $t=0$ in any of the 3 treatment groups ($P=0.8175$ and $P=0.1223$, respectively). Already after 3 months of ERT, independent of the treatment regimen, a significant reduction ($P=0.0313$) in plasma lysoGb3 concentration was reached. Thereafter lysoGb3 levels did not decrease further up to 12 months of treatment (*P*-values for each treatment regimen >0.05; see also Fig. 2, Table 1). Reductions in plasma Gb3 by ERT were noted within 3 months of ERT (*P*-values for each treatment regimen <0.01). Comparison of the changes in plasma lysoGb3 (or Gb3) concentration induced by the 3 different treatments revealed no clear difference (see Fig. 2). The use of repeated measures analysis of variance revealed that the reduction in plasma lysoGb3 was significantly larger for patients treated with agalsidase beta at 1.0 mg/kg compared with patients treated with agalsidase alfa ($P=0.003$) and beta ($P=0.046$) at a dose of 0.2 mg/kg.

3.3. Formation of anti-(α-Gal A) antibodies and impact on plasma lipid reduction

We examined the influence of the treatment regimens on formation of neutralizing antibodies. Table 2 shows that 16 of the 22 hemizygotes had detectable antibodies. The lowest percentage of antibody formation (43%) was noted for patients receiving agalsidase alfa. The dose of agalsidase beta did not seem to strongly determine the chance on antibody formation in hemizygotes (0.2 mg/kg: 83%, 1.0 mg/kg: 89%). None of the female patients developed anti-(α-Gal A) antibodies.

The impact of the presence of neutralizing antibodies on ERT-induced reductions in plasma lysoGb3 and Gb3 was examined. Plasma lysoGb3 and Gb3 levels at $t=0$ were comparable in hemizygotes, whether developing antibodies during ERT or not ($P=0.0972$ and $P=0.3568$, respectively). Plasma lysoGb3 concentrations tended to be higher in hemizygotes with antibodies when treated for 12 months with agalsidase alfa or beta at a dose of 0.2 mg/kg, compared with hemizygotes without antibodies[1] (Fig. 3). This was not the case for patients treated for

[1] Plasma lysoGb3 and Gb3 concentrations of male patients were not significantly different at $t=0$ in any of the 4 groups described in Fig. 3 ($P=0.2505$ and $P=0.2088$, respectively).

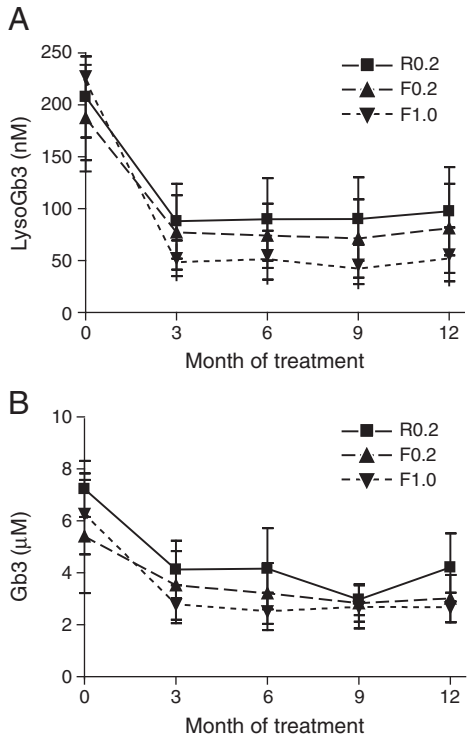

Fig. 2. Effect of different treatment regimens on plasma lipid levels in male Fabry patients. (A) Plasma lysoGb3 levels in male Fabry patients prior to therapy and after 3, 6, 9 and 12 months of therapy. (B) Plasma Gb3 levels in male Fabry patients prior to therapy and after 3, 6, 9 and 12 months of therapy. R0.2, treatment with agalsidase alfa at 0.2 mg/kg; F0.2, treatment with agalsidase beta at 0.2 mg/kg; F1.0, treatment with agalsidase beta at 1.0 mg/kg. Data are presented as mean (and sd) values at the indicated time points for each group.

12 months with agalsidase beta at a dose of 1.0 mg/kg[1] (Fig. 3). The same picture emerges when analyzing lipid data of individuals expressed as percentage of $t=0$ (pre-treatment) using repeated measures analysis of variance: the reduction in plasma lysoGb3 is significantly less for patients with antibodies receiving a dose of 0.2 mg/kg agalsidase alfa ($P=0.008$) compared to those without antibodies. The effect was less for patients with antibodies receiving a dose of 0.2 mg/kg agalsidase beta ($P=0.192$). Of note, this analysis indicated that the plasma lysoGb3 reduction in patients receiving a dose of 0.2 mg/kg agalsidase alfa was just not significantly different from that in patients receiving a dose of 0.2 mg/kg agalsidase beta ($P=0.082$). The lysoGb3 reduction in patients receiving a dose of 1.0 mg/kg agalsidase beta was comparable to that in patients without antibodies ($P=0.497$).

3.4. Effect of different ERT regimens on plasma lipids in heterozygotes

Finally, we investigated the response of plasma lipid concentrations in heterozygotes to different treatment regimens. Plasma lysoGb3 and Gb3 concentrations of female patients were not significantly different at $t=0$ in any of the 3 treatment groups ($P=0.5398$ and $P=0.4138$, respectively; see also Table 3). During ERT plasma Gb3 concentrations hardly changed, which is not surprising given the fact that these are mostly within the normal range (upper limit of normal range 3.18 μM). In female patients, plasma lysoGb3 concentrations at $t=0$ were rather low, compared with males. The range of plasma lysoGb3 concentrations was large in some of the treatment groups. One female patient showed lysoGb3 levels below the detection limit at start of ERT whereas another heterozygote, also receiving 1.0 mg/kg agalsidase beta, showed a relatively high lysoGb3 (143 nM). Thus, a direct comparison of the effect of different treatments on plasma lysoGb3 in female patients is difficult given the heterogeneity among the studied patients; however most patients ($n=17$) showed a reduction with ERT. A minority ($n=4$) showed stabilization at low level with ERT. None of the female patients showed an increase in plasma lysoGb3 with ERT.

4. Discussion

ERT of Fabry disease is extremely costly and demonstration of its clinical efficacy is difficult. Reductions in the storage lipid Gb3 in skin, kidney and cardiac biopsies have been documented earlier [29], substantiating the ability of recombinant α-Gal A preparations to effectively supplement cells of Fabry patients with degradative capacity. Unfortunately, plasma Gb3 concentration has not been found to be a useful surrogate marker and the use of sequential plasma Gb3 measurements for monitoring efficacy of ERT has not been recommended [26,27]. Recently it has become clear that plasma lysoGb3 elevation is a characteristic feature of patients with classical manifestations of Fabry disease [28]. The increase in the plasma concentration of this lipid is most striking in hemizygotes. The relative extent of the increase in plasma concentration of lysoGb3 in Fabry patients is far larger than that of Gb3. The aim of our investigation was to establish whether plasma lysoGb3 concentrations of Fabry patients are changed by ERT, and may have potential as a surrogate marker to monitor therapeutic intervention. For this purpose, patients with classical

Table 1
Effect of different treatment regimens on plasma levels of lysoGb3 and Gb3 in male Fabry patients. The data reflect absolute numbers (median and range) and sample size (n). R0.2, treatment with agalsidase alfa at 0.2 mg/kg; F0.2, treatment with agalsidase beta at 0.2 mg/kg; F1.0, treatment with agalsidase beta at 1.0 mg/kg. Normal values for lysoGb3 <3 nM, normal values for Gb3 <3.18 μM.

Males	LysoGb3 (nM)								
	$t=0$			$t=3$			$t=12$		
	Median	Range	n	Median	Range	n	Median	Range	n
Total	192	(102–397)	22	65	(20–136)	18	61	(16–149)	22
R0.2	220	(148–250)	7	86	(46–136)	6	118	(52–149)	7
F0.2	192	(102–263)	6	87	(20–120)	6	96	(16–131)	6
F1.0	189	(134–397)	9	52	(33–76)	6	55	(23–113)	9
Males	Gb3 (μM)								
	$t=0$			$t=3$			$t=12$		
	Median	Range	n	Median	Range	n	Median	Range	n
Total	6.52	(3.31–9.10)	22	3.22	(1.72–5.50)	18	3.17	(1.34–6.42)	22
R0.2	7.03	(5.95–8.80)	7	4.31	(2.74–5.29)	6	3.74	(3.07–6.42)	7
F0.2	4.91	(3.31–9.10)	6	3.08	(2.22–5.50)	6	3.38	(1.85–3.93)	6
F1.0	6.56	(3.97–8.74)	9	2.91	(1.72–3.57)	6	2.77	(1.34–3.37)	9

Table 2
Antibody formation in male Fabry patients treated with different treatment regimens. The data reflect absolute and relative (%) numbers. R0.2, treatment with agalsidase alfa at 0.2 mg/kg; F0.2, treatment with agalsidase beta at 0.2 mg/kg; F1.0, treatment with agalsidase beta at 1.0 mg/kg. AB+, patients with neutralizing antibodies; AB−, patients without neutralizing antibodies.

Males	Total	AB+	AB−	% AB+
Total	22	16	6	73
R0.2	7	3	4	43
F0.2	6	5	1	83
F1.0	9	8	1	89

manifestations of Fabry disease were examined that were treated by two weekly intravenous administration of agalsidase alfa at 0.2 mg/kg, agalsidase beta at 0.2 mg/kg, or agalsidase beta at 1.0 mg/kg.

In all investigated male patients plasma lysoGb3 was high prior to ERT (102–397 nM; control subjects: <3 nM). The 3 different treatment regimens all resulted in marked reductions in lysoGb3 within 3 months, after which the levels tended to stabilize. No clear differences were noted between the different treatments. Examination of the occurrence of anti-(α-Gal A) antibodies, determined by an *in vitro* neutralizing assay, revealed a high incidence, particularly among patients receiving agalsidase beta. Treatment with a high dose agalsidase beta led to a similar reduction in plasma lysoGb3 as observed in patients without antibodies. This suggests that a dose increase can compensate for reduced efficacy of recombinant α-Gal A in individuals with anti-(α-Gal A) antibodies. Negative effects of antibodies on ERT-induced reductions in urinary Gb3 were reported earlier, which also could be compensated by the use of high dose enzyme infusion [36–38].

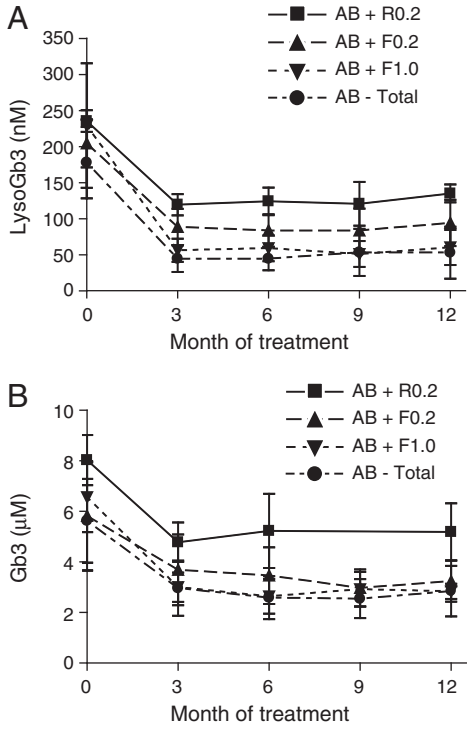

Fig. 3. Effect of different treatment regimens on plasma lipid levels in male Fabry patients with and without anti-(α-Gal A) antibodies. (A) Plasma lysoGb3 levels in male Fabry patients with (AB+) and without (AB−) anti-(α-Gal A) antibodies prior to therapy and after 3, 6, 9 and 12 months of therapy. (B) Plasma Gb3 levels in male Fabry patients with and without anti-(α-Gal A) antibodies prior to therapy and after 3, 6, 9 and 12 months of therapy. R0.2, treatment with agalsidase alfa at 0.2 mg/kg; F0.2, treatment with agalsidase beta at 0.2 mg/kg; F1.0, treatment with agalsidase beta at 1.0 mg/kg. Data are presented as mean (and sd) values at the indicated time points for each group.

In female patients, plasma lysoGb3 levels are much lower than in male patients. A direct comparison of the effect of different treatments on plasma lysoGb3 concentration was hampered by the relatively large variability in its concentration in the investigated females prior to treatment. However, it is noteworthy that in all heterozygotes, with each of the treatment regimen, a reduction in plasma lysoGb3 level or at least stabilization at low level was observed. None of the female patients developed anti-(α-Gal A) antibodies.

The high plasma concentration of lysoGb3 is characteristic for patients with classical manifestations of Fabry disease. A role for lysoGb3 in pathogenesis of Fabry disease, as suggested for deacylated galactosylceramide in Krabbe disease [42–44], is presently under investigation. Of note, at concentrations occurring in plasma of hemizygotes, lysoGb3 has been found to promote smooth muscle cell proliferation [28]. In addition, lysoGb3 has been reported to stimulate cultured human podocytes to express TGF-β1, extracellular matrix proteins (fibronectin and type IV collagen) and the macrophage inhibitory factor receptor CD74 [45]. These factors have been implicated in glomerular injury in diabetic nephropathy. Recent data suggest that high plasma lysoGb3 correlates with increased risk for cerebrovascular disease in Fabry males and LVH in Fabry females. Lifetime exposure to lysoGb3, assessed as plasma lipid concentrations multiplied by age, was found to correlate with disease severity in male as well as female patients [41]. These observations suggest, but certainly do not prove, that lysoGb3 plays a direct role in the pathogenesis of Fabry disease. Of note, only partial corrections in plasma lysoGb3 concentrations of male patients receiving ERT were observed. Apparently, even high dose ERT is insufficient to completely correct the plasma lysoGb3 abnormality. Thus, it might be possible that ERT of male Fabry patients results in an ameliorated progression of disease and not a complete halt or reversal of disease manifestation. We noted no correlation of plasma lysoGb3 content of male Fabry patients with disease manifestation prior to, or after one year of therapy. Among female Fabry patients, the combined disease severity score (Mainz severity scoring index (MSSI) [46]) showed no significant correlation with plasma lysoGb3 levels prior to treatment (Spearman ρ 0.4421; $P=0.099$). Changes in plasma lysoGb3 within one year of ERT were no obvious predictor for changes in some clinical parameter.

At present plasma lysoGb3 does not qualify as surrogate marker of Fabry disease. Long-term follow-up of Fabry patients receiving ERT has to reveal whether plasma lysoGb3 levels correlate with meaningful clinical outcomes such as progression of cardiac, renal or

Table 3
Effect of different treatment regimens on plasma levels of lysoGb3 and Gb3 in female Fabry patients. The data reflect absolute numbers (median and range) and sample size (n). R0.2, treatment with agalsidase alfa at 0.2 mg/kg; F0.2, treatment with agalsidase beta at 0.2 mg/kg; F1.0, treatment with agalsidase beta at 1.0 mg/kg. Normal values for lysoGb3 <3 nM, normal values for Gb3 <3.18 μM.

Females	LysoGb3 (nM)					
	$t=0$			$t=12$		
	Median	Range	n	Median	Range	n
Total	14	(0–143)	21	8	(0–35)	21
R0.2	23	(12–26)	7	13	(6–19)	7
F0.2	11	(7–29)	5	8	(6–30)	5
F1.0	8	(0–143)	9	5	(0–35)	9
Females	Gb3 (μM)					
	$t=0$			$t=12$		
	Median	Range	n	Median	Range	n
Total	2.61	(0.77–6.76)	21	2.11	(0.79–3.65)	20
R0.2	2.82	(2.45–3.28)	7	2.44	(2.02–3.60)	7
F0.2	2.34	(0.77–4.18)	5	2.48	(1.40–3.01)	5
F1.0	2.53	(1.20–6.76)	9	1.76	(0.79–3.65)	8

cerebrovascular complications. It will be of particular interest to analyze whether a poor response in plasma lysoGb3 following ERT is associated with a poor clinical response. Since it has become clear that advanced disease cannot be reversed by ERT, correction of lysoGb3 is presumably of little value in this group. However, when early treatment has been installed, measurement of plasma lysoGb3 concentration may prove to be a valuable tool in clinical management of Fabry patients before irreversible damage has occurred.

In conclusion, our investigation has rendered a valuable new insight. ERT of patients with classical manifestations of Fabry disease with recombinant α-Gal A preparations results in correction of plasma lysoGb3. This finding further substantiates that ERT is capable of correcting a characteristic biochemical abnormality in Fabry patients.

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